

Physiological aspects of pig-to-primate renal xenotransplantation

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Background. Few data exist on the physiological aspects of pig-to-primate renal xenotransplantation.

Methods. Use of organs transgenic for human decay accelerating factor has allowed assessment of the metabolic and hormonal functions of these xenografts.

Results. Porcine renal xenografts largely maintain plasma electrolyte homeostasis. An increase in proteinuria was detected that may result from graft injury. In contrast to allotransplantation a severe anaemia developed requiring recipient treatment with exogenous human erythropoietin.

Conclusions. Our experience provides qualified encouragement for the likely physiological compatibility of pig and primate species, but identifies areas where a xenograft may not match the performance of an allograft.

Xenotransplantation research has focused on the immunological response to porcine tissue and the risk of zoonotic infection. Physiological compatibility between species has received less attention because of short survival times in the pig-to-nonhuman primate model [1–3].

Although porcine insulin can successfully control human diabetes, some other hormones fail to function across species. Porcine renin does not cleave human angiotensinogen, and growth hormone activity is not necessarily conserved between species [4–6]. Thus, physiological incompatibilities may have important implications for pig-to-human renal transplantation, which is a potential solution to the shortfall of donor organs.

The use of porcine organs, transgenic for human regulators of complement activity (in particular decay accelerating factor, CD55), in combination with immunosup-

pressive therapy has enabled prolonged survival times of over two months in life-supporting models of pig-to-primate renal xenotransplantation [7, 8]. For much of this time, the animals are physically well, with extended periods of rejection-free survival. This increased survival has allowed the study of the metabolic and hormonal effects of discordant renal xenotransplantation, which are reported here.

METHODS

Human decay accelerating factor transgenic pig-to-primate renal xenotransplantation

The human decay accelerating factor (hDAF) transgenic pig-to-cynomolgus monkey renal xenotransplantation model with bilateral native nephrectomies has been established in our unit [7]. Kidneys from hDAF transgenic piglets (aged 22 to 36 days) were transplanted into cynomolgus monkeys (aged 18 months to 3 years) in accordance with the Animals (Scientific Procedures) Act 1986. All recipients received induction immunosuppression that consisted of cyclosporine A (Neoral®), cyclophosphamide (CyP), and corticosteroids as described previously by our group [8]. A further maintenance immunosuppressive agent also was administered in combination with continued cyclosporine A and reducing doses of oral corticosteroids. The single agent used was one of the following: CyP, mycophenolate mofetil (MMF), ERL (an enteric-coated formulation of mycophenolate sodium), or RAD (an immunosuppressive macrolide, 40-O-hydroxymethyl-rapamycin). In some studies, recipient splenectomy was also performed at the time of transplantation as part of the immunosuppressive therapy.

Measurements of electrolytes and protein

Daily serum electrolytes were monitored in 22 animals that survived at least 20 days (range of 21 to 78 days, mean survival of 41 days, median survival of 38 days)

Key words: allograft, xenograft, renal transplantation, zoonotic infection, cross-species transplant, immunosuppression therapy, organ donation, interspecies physiology.

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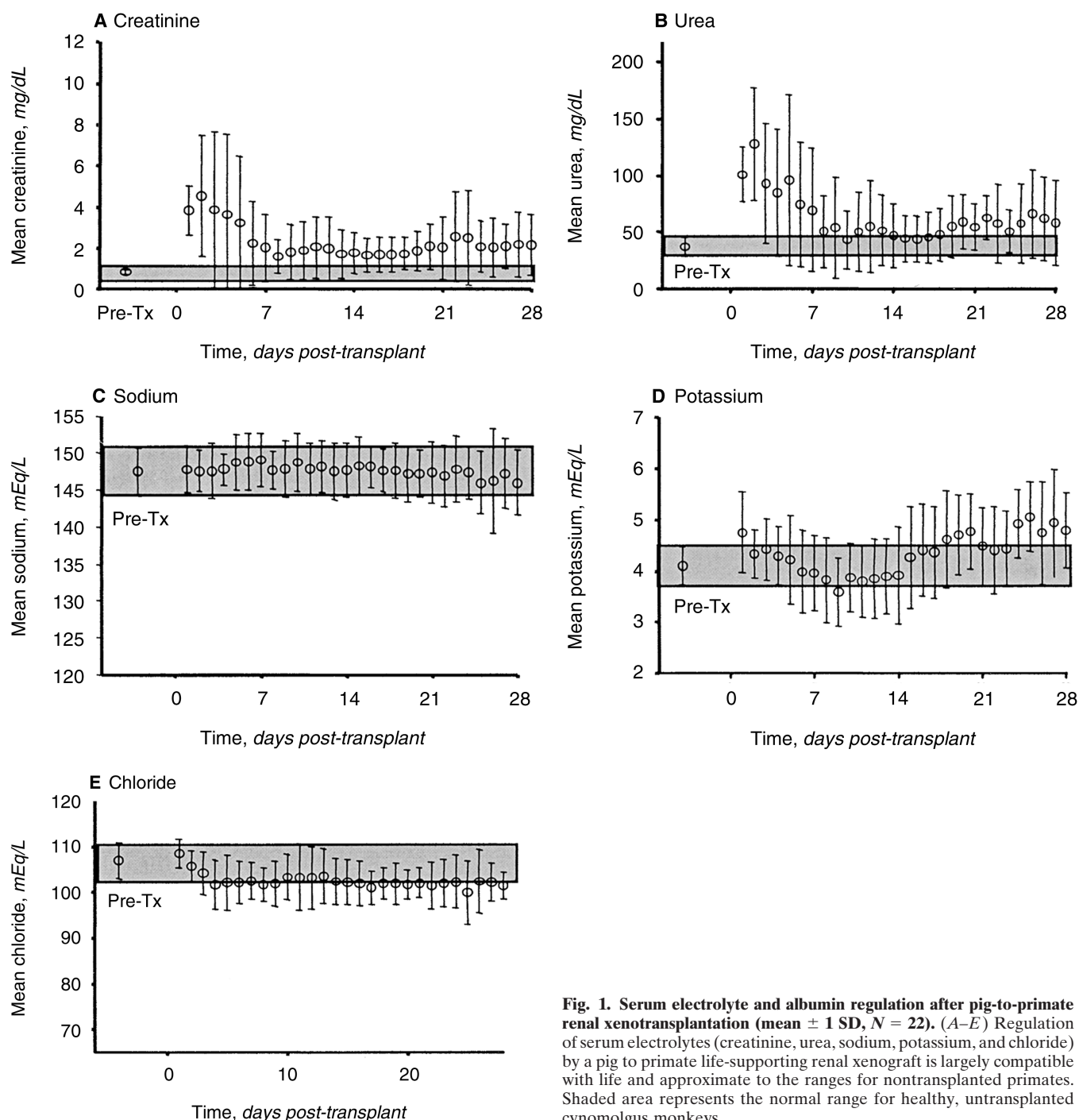


Fig. 1. Serum electrolyte and albumin regulation after pig-to-primate renal xenotransplantation (mean \pm 1 SD, $N = 22$). (A–E) Regulation of serum electrolytes (creatinine, urea, sodium, potassium, and chloride) by a pig to primate life-supporting renal xenograft is largely compatible with life and approximate to the ranges for nontransplanted primates. Shaded area represents the normal range for healthy, untransplanted cynomolgus monkeys.

using a Hitachi 737 Clinical Chemistry analyzer. Normal ranges for hematological and biochemical parameters for cynomolgus monkeys were based on data provided by an animal reference laboratory, collected on more than 500 untreated animals over a ten-year period. Animals were treated with enteral and parenteral fluid and potassium solutions as clinically indicated. This was particularly important in the management of some animals, which developed severe diarrhea.

Analysis of urine collected from cynomolgus monkey recipients ($N = 7$) were performed on alternate days post-transplantation. The collections were made overnight for 8- to 12-hour periods. The animals were housed singly during the collections. Urine was collected via a gravitational system that allowed filtering of solid matter. After centrifugation of the sample, urinary electrolytes and protein were measured on a Hitachi 917 analyzer. Albumin and globulin fractions were quantified using

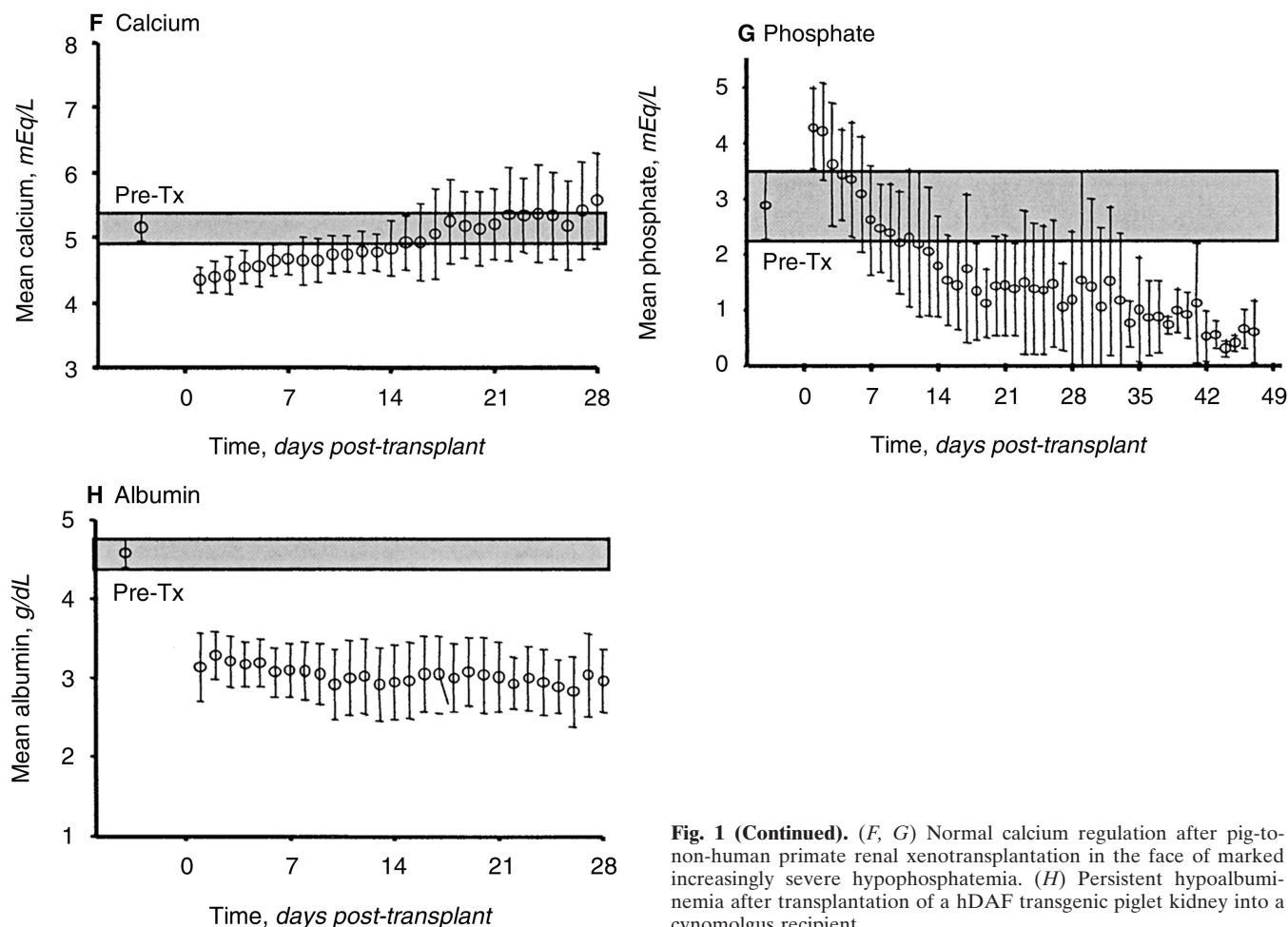


Fig. 1 (Continued). (F, G) Normal calcium regulation after pig-to-non-human primate renal xenotransplantation in the face of marked increasingly severe hypophosphatemia. (H) Persistent hypoalbuminemia after transplantation of a hDAF transgenic piglet kidney into a cynomolgus recipient.

gel electrophoresis. Ten samples were collected from the recipients preoperatively and 23 during the subsequent postoperative course after day 3, once the initial period of graft dysfunction post-transplantation had started to recover.

Hemoglobin and erythropoietin

In 16 animals, hematological analyses were performed using a Bayer-Technicon H1E hematology analyzer. The first seven animals became severely anemic, and subsequent animals received preoperative and postoperative treatment with exogenous recombinant human erythropoietin (rhEpo). At this time, splenectomy was introduced as an additional form of immunosuppression.

RESULTS

General condition and animal activity

After recovery from the transplantation procedure, the animals ate normally and showed normal behavior and activity. Experiments were usually ended because of the development of renal failure or complications of

immunosuppression in the recipient. In animals with good renal xenograft function, there was no evidence of fluid retention.

Measurement of electrolytes and protein

Following an initial period of postoperative graft dysfunction, plasma urea, sodium, chloride, and potassium remained largely within normal limits while the animal remained well. Plasma creatinine reduced after the same period of initial graft dysfunction, but the mean values remained higher than the normal range for non-transplanted primates (Fig. 1 A–E). In animals with good renal function, normal fluid balance and body weight were maintained. Animals with renal failure tended to develop ascites and peripheral edema.

Urine collection volumes for the two groups, pretransplantation and post-transplantation, were not statistically different (pretransplant volume 29.3 ± 35 mL; post-transplant volume 44.2 ± 19 mL, $P = 0.24$). After xenotransplantation, the concentration of potassium in the urine increased compared with that prior to operation, but this was not statistically significant (pretransplant

urinary potassium 1.3 ± 1.6 mEq/L; post-transplant urinary potassium 2.1 ± 1.5 mEq/L, $P = 0.8$). The recipients required enteral and parenteral potassium supplementation on approximately half of the postoperative days. Treatment with potassium supplements and intravenous fluids was usually required when the animals had excess gastrointestinal losses due to diarrhea. The recipient animals were observed to pass loose or liquid feces on approximately half of the postoperative days. The diarrhea was usually not infective in origin but secretory or malabsorptive diarrhea related to the immunosuppressive therapy. The ability to concentrate the urine was preserved (pretransplantation urinary osmolarity 296 ± 133 mOsm/kg, post-transplantation urinary osmolarity 405 ± 102 mOsm/kg, $P = 0.22$). There was an increase in sodium excretion (pretransplant urinary sodium 0.94 ± 1.31 mEq/L, post-transplant urinary sodium 3.3 ± 2.4 mEq/L, $P = 0.03$). It has been previously noted in the veterinary literature that there are relatively higher amounts of sodium excreted in the urine of young piglets [9]. Plasma calcium was maintained at near normal levels after transplantation without exogenous supplementation (Fig. 1F). However, plasma phosphate levels fell continuously after renal xenotransplantation in all animals studied (Fig. 1G). Hypoalbuminemia after transplantation was a consistent finding in all animals (Fig. 1H).

Proteinuria increased fourfold following transplantation (pretransplantation 14.7 ± 18.2 mg/dL, post-transplantation 85.7 ± 48.6 mg/dL, $P < 0.01$) and was characterized as a nonselective loss of both globulins and albumin by electrophoresis. The urinary protein concentration did not correlate with any deterioration in renal function or with anti-pig hemolytic antibody titers, a marker of the humoral anti-xenograft immune response by the recipient (data not shown). In the two longest survivors, urinary albumin concentration increased as renal function declined and antibody titers increased, but this pattern was not apparent in animals surviving for shorter times.

Hemoglobin and erythropoietin

Animals in the nonsplenectomized, non-rhEpo-treated group became progressively anemic, necessitating termination of the experiment in the four longest survivors. The anemia was normocytic and normochromic with normal plasma bilirubin, haptoglobin, and peripheral blood films (data not shown). There was no evidence of hemolysis. Reticulocyte counts were low ($<0.1\%$ for the first 3 weeks). In the rhEpo-treated group, there was a decline in hemoglobin during the first post-operative week as a consequence of surgical blood losses, immunosuppression, and blood sampling, followed by a marked reticulocytosis and subsequent return of the hemoglobin to near

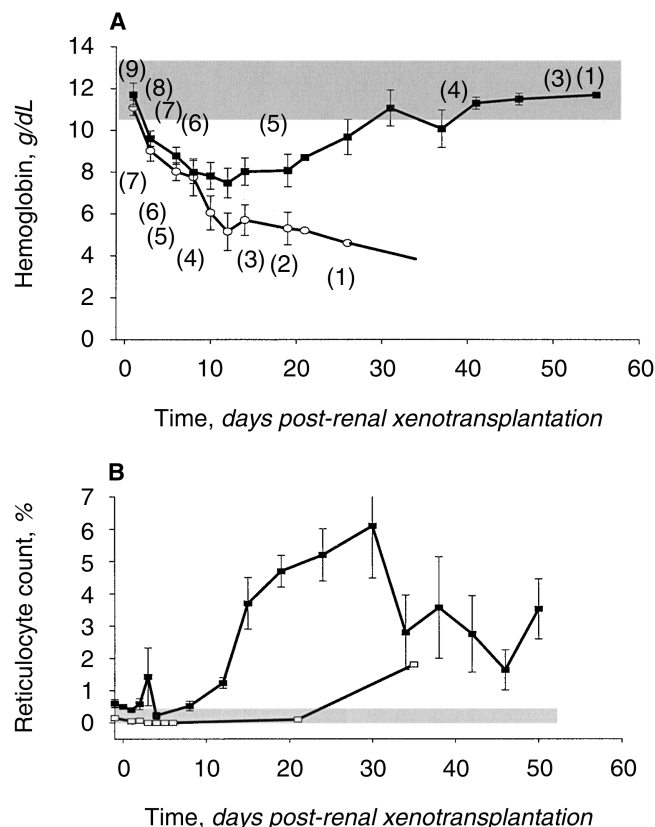


Fig. 2. (A) Mean (± 1 SD) hemoglobin in erythropoietin (Epo; ■) and non-Epo (□) treated monkey recipients of transgenic pig kidney xenografts. The anemia responsive to treatment with exogenous recombinant human Epo (rhEpo) treatment. The normal range is shaded. Numbers of animals analyzed at each time point are given in parentheses. (B) Reticulocyte response to anemia is restored by exogenous rhEpo administration after pig to non-human primate renal xenotransplantation (non-Epo, $N = 7$; Epo, $N = 9$). The shaded area represents the normal range for healthy, untransplanted cynomolgus monkeys.

normal values (Fig. 2). Although this group also had splenectomy performed, our subsequent experience with this model suggests that splenectomy does not cause an increased tendency to anemia or improve the hemopoietic response after renal xenotransplantation.

DISCUSSION

Our studies now show that transgenic porcine kidney xenografts can support life in primate recipients. The maintenance of body water and electrolyte homeostasis, except for phosphate, suggests interspecies physiological compatibility of the controlling hormone systems. This regulation occurs in spite of some differences in electrolyte handling that may be model-specific. For example, it has been previously noted in the veterinary literature that there are relatively higher amounts of sodium excreted in the urine of young piglets [9].

We have not performed an in-depth study of the actions of primate hormones on the porcine xenograft.

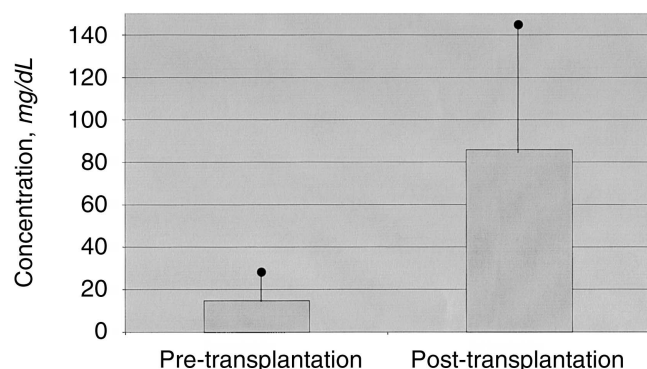


Fig. 3. Proteinuria before and after renal xenotransplantation. Each graph demonstrates the increase in urinary protein concentration, mainly albumin and globulins, after pig-to-cynomolgus renal xenotransplantation.

However, we can infer that certain functions are preserved. Active renin is a glycoprotein acid protease exclusively secreted by the kidney [10]. The fact that porcine renin fails to cleave human or nonhuman primate angiotensinogen in vitro might be expected to result in hyperkalemia due to secondary hypoaldosteronism. The renin-angiotensin-aldosterone axis may be at least in part maintained in vivo because serum potassium concentration in recipients of porcine renal xenografts was within the normal range or below it, sometimes necessitating supplementation. The increased urine osmolarity demonstrates an ability to concentrate urine, which implies that ADH is functional between species in this model. However, the anemia, which can be corrected by exogenous rhEpo, suggests a failure of interspecies hormone action. Transient and usually mild hypophosphatemia is common following allogeneic renal transplantation. The profound, prolonged hypophosphatemia seen after renal xenotransplantation suggests that the porcine renal tubules may either be unresponsive to primate vitamin D or have an increased response to parathyroid hormone or be injured, all of which might contribute to the hypophosphatemia.

Increased proteinuria was observed (Fig. 3) and may be indicative of renal injury after xenotransplantation or abnormal handling by the pig kidney of primate plasma proteins. The protein loss consisted of albumin and globulins. Proteinuria also has been described by other scientists using a pig-to-baboon renal xenotransplantation model, although survival in their recipients was much shorter, as was the period of rejection free function (abstract; Cohen et al, *Poster Presented at 5th International Xenotransplantation Congress*, Nagoya, Japan, October 1999).

Proteinuria after renal allotransplantation is not usual and is associated with defects in glomerular charge and size selectivity, secondary to pathological states such as rejection or recurrent glomerulonephritis [11–13]. Con-

tinuous, but not intermittent, proteinuria is associated with reduced graft survival [14, 15]. Environmental and physical stress can increase proteinuria in adult pigs [16, 17], and proteinuria is often observed in neonatal piglets [18, 19], due to a limited ability of the immature proximal tubules to reabsorb filtered protein [20]. Complement-mediated glomerular injury is also associated with proteinuria [21, 22]. Ongoing complement-mediated injury after renal xenotransplantation, along with the limited capacity of the young donor kidney to reabsorb filtered protein, may explain the proteinuria found in this model [23].

The observed anemia is most likely to be due to a failure of Epo action, as cardiac xenotransplantation carried out with the same donor and recipient combinations and immunosuppressive regimens did not develop anemia (unpublished data from our unit). This was supported by the lack of laboratory evidence of hemolysis.

Possible mechanisms for the erythropoietic failure include insufficient Epo production by the juxtaglomerular cells of the renal xenograft. In spite of greater than 80% amino acid identity between porcine and non-human primate Epos, failure of porcine Epo to activate the primate Epo receptor in the bone marrow may also be a factor. Indeed, certain amino acid replacements can alter important structural bonds and key areas have been identified in vitro where amino acid substitutions reduce hormone activity and Epo receptor affinity [24, 25].

A neutralizing antibody response against porcine Epo seems unlikely, as porcine Epo does not appear to express the Gal α 1,3-Gal β 1GlcNAc-R epitope and the anemia occurs immediately, before a de novo antibody response would be expected, particularly in the context of profound immunosuppression (abstract; Soin et al, *Ann Haematol* 79:B7, 2000).

Measurements of circulating serum Epo were performed using commercially available ELISA kits, but our group could not validate these assays as either sensitive to or specific for porcine Epo. Further work is required to prove that any detectable erythropoietin is derived from the xenografted porcine kidney rather than primate Epo from extrarenal sites, such as the liver.

Pig kidneys transplanted into primates can support healthy life in the recipient, when free from rejection. Exceptions to normal homeostasis are a failure of Epo action, proteinuria, and severe hypophosphatemia. Adjuvant therapy such as exogenous rhEpo administration or the use of phosphate supplements may ameliorate the effects of these apparent regulatory failures. Proteinuria may be a model-specific problem or be caused by pathological glomerular injury. Given these exceptions, our experience in a life-supporting model of renal xenotransplantation, with survival for up to 78 days, provides qualified encouragement regarding the likely physiological compatibility of pig and primate species.

Extended survival of rejection-free renal xenografts beyond that reported here might not be possible in a model using a single transgenic manipulation and immunosuppressive agents. Success with other strategies such as the production of α -Gal knockout pigs or tolerance induction may allow disease free periods of 6 to 12 months over which the inter-species physiological compatibility can be further assessed. In vitro models may be constructed to identify further the cellular receptor mechanisms underlying erythropoietic failure, proteinuria, and hypophosphatemia. However, because of the complex interactions between the many different neurohormonal systems in the body further in vivo studies (or clinical xenotransplantation) will be the real test of whether porcine organs are a viable alternative to human allografts.

The decision to proceed with clinical trials will be based on the relative risks and benefits to the patient and the general population. This research demonstrates areas in which the performance of porcine donor organs may not match those of an allograft. These factors must be included in any assessment of the potential benefit of xenotransplantation against the potential risks of infection and the known side effects of the required immunosuppression.

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